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(54) Thermostable luciferase of a firefly, gene of a thermostable luciferase of a firefly, novel recombinant DNA, and process for the preparation of a thermostable luciferase of a firefly.

(57) The present invention relates to a thermostable luciferase of a firefly wherein an amino acid at the 217-position of the amino acid sequence of the wild-type firefly luciferase or an amino acid equivalent to the amino acid at the 217-position of the luciferase of GENJI firefly or HEIKE firefly is converted into a hydrophobic amino acid, a gene encoding said thermostable luciferase, a vector comprising the gene encoding said thermostable luciferase inserted therein, and a process for the preparation of a thermostable firefly luciferase comprising the use of said vector.

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(1)

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(71) Applicant (*for all designated States except US*): PROMEGA CORPORATION [US/US]; 2800 Woods Hollow Road, Madison, WI 53711-5399 (US).

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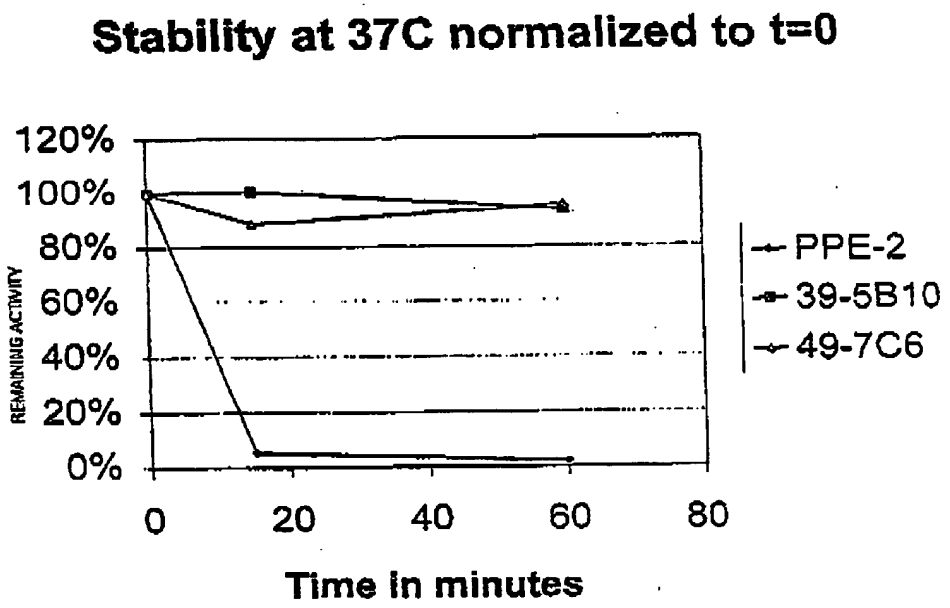
(54) Title: THERMOSTABLE LUCIFERASES AND METHODS OF PRODUCTION



(57) Abstract

Luciferase enzymes with greatly increased thermostability, e.g., at least half lives of 2 hours at 50 °C, cDNAs encoding the novel luciferases, and hosts transformed to express the luciferases, are disclosed. Methods of producing the luciferases include recursive mutagenesis. The luciferases are used in conventional methods, some employing kits.

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(2)

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09/396,154 15 September 1999 (15.09.1999) US

(71) Applicant: PROMEGA CORPORATION [US/US]; 2800 Woods Hollow Road, Madison, WI 53711 (US).

(72) Inventors: WOOD, Keith, V.; 902 Kottke Drive, #5, Madison, WI 53719 (US). HALL, Mary, P.; 6305 Dylyn Drive, Madison, WI 53719 (US). GRUBER, Monika; 1312 Drake Street, Madison, WI 53715 (US).

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(81) Designated States (national): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

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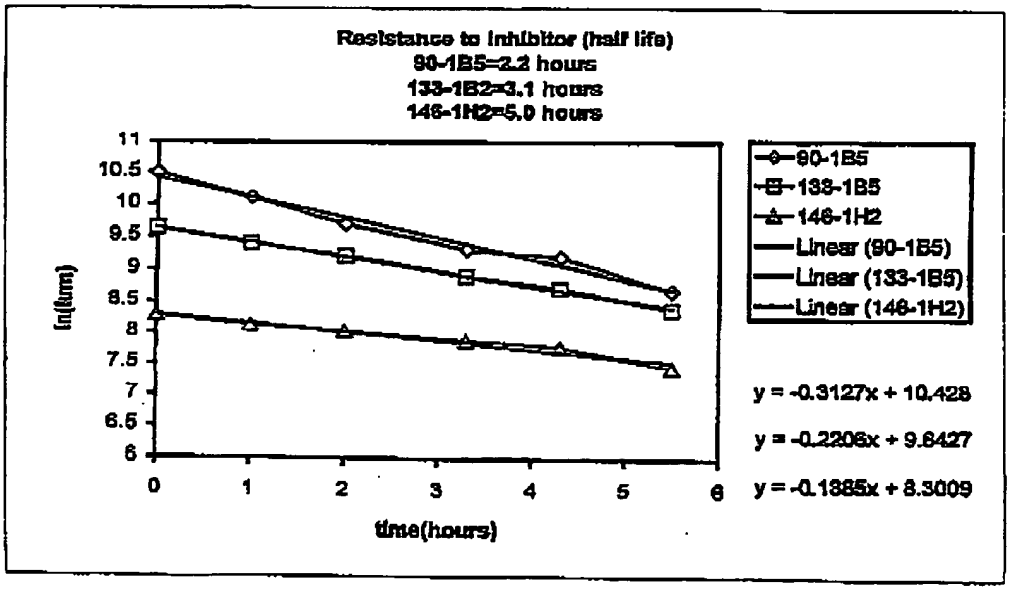
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: THERMOSTABLE LUCIFERASES FROM PHOTURIS PENNSYLVANICA AND PYROPHORUS PLAGIOPHTHALAMUS AND METHODS OF PRODUCTION



(57) Abstract: Luciferase enzymes with greatly increased thermostability, e.g., at least half lives of 2 hours at 50 °C, cDNAs encoding the novel luciferases, and hosts transformed to express the luciferases, are disclosed. Methods of producing the luciferases include recursive mutagenesis. The luciferases are used in conventional methods, some employing kits.

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: NOVEL ENZYME

(57) Abstract

A protein having luciferase activity and at least 60 % similarity to luciferase from *Photinus pyralis*, *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis*, *Hotaria paroula*, *Pyrophorus plagiophtalamus*, *Lampyris noctiluca*, *Pyrocoelia nayako* or *Photinus pennsylanvanica* wherein in the sequence of the enzyme, at least one of (a) the amino acid residue corresponding to residue 214 in *Photinus pyralis* luciferase; (b) the amino acid residue corresponding to residue 232 in *Photinus pyralis* luciferase; (c) the amino acid residue corresponding to residue 295 in *Photinus pyralis* luciferase; (d) the amino acid residue corresponding to acid 14 of *Photinus pyralis* luciferase; (e) the amino acid residue corresponding to amino acid 35 of *Photinus pyralis* luciferase; (f) the amino acid residue corresponding to amino acid residue 105 of *Photinus pyralis* luciferase; (g) the amino acid residue corresponding to amino acid residue 234 of *Photinus pyralis* luciferase; (h) the amino acid residue corresponding to amino acid residue 420 of *Photinus pyralis* luciferase; (i) the amino acid residue corresponding to amino acid residue 310 of *Photinus pyralis* luciferase: is different to the amino acid which appears in the corresponding wild type sequence and wherein the luciferase enzyme has increased thermostability as compared to an enzyme having the amino acid of the corresponding wild-type luciferase at this position.

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: MUTANT LUCIFERASE

(57) Abstract: A recombinant protein having luciferase activity and at least 60 % similarity to a wild-type luciferase wherein in the sequence of the enzyme, the amino acid residue corresponding to residue 357 in *Photinus pyralis* luciferase is mutated as compared to the corresponding wild-type luciferase, such that the luciferase enzyme is able to emit light at a different wavelength as compared to the corresponding wild-type luciferase and/or has enhanced thermostability as compared to the corresponding wild-type luciferase. In general, the residue corresponding to 357 in Photinus pyralis luciferase is changed from an acidic amino acid to a non-acidic amino acid and preferably an uncharged polar amino acid such as tyrosine. Mutant luciferases in accordance with the invention can produce a large (50nm) wavelength shift in emitted light and have good thermostability. The resultant colour shift can be reversed by addition of coenzyme A. These properties make the mutant particularly useful in a variety of assays.